THE UPTAKE OF VANADIUM BY TUNICATES[1]

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The ability of certain ascidians to concentrate vanadium was first reported in 1911 by Henze (1911), who found this element in the blood of four species. Since then more than a score of papers have extended these investigations.[2] The scope of the above work has included the analyses of various species of ascidians to determine the presence or absence of vanadium, and experiments to indicate any possible physiological function of the vanadium.

The first phase of these studies culminated in the work of Webb (1939) who was able to correlate the presence of vanadium in ascidians with certain evolutionary traits of the class. Webb concluded that vanadium is concentrated by the primitive species and that this enrichment ability has been lost in the later, more specialized, branches of the class.

Webb also systematically studied the physiological role of vanadium. He demonstrated that the green chromogen containing the vanadium in some species is located in a singular type of blood cell which he called the "vanadocyte." Later, however, in histological investigations on certain Japanese ascidians, Ohuye (1936) described what may be the same type of green cells in these animals which, according to the analyses of Kobayashi (1948), contained no vanadium. Webb found no physiological function for the vanadium, although previously it had been assumed to be associated with a blood respiratory pigment. He also postulated that the vanadium was incorporated in a rectilinear chain of pyrrole rings rather than in a protein or porphyrin complex.

The reported distribution of the vanadium within ascidians is somewhat contradictory. Webb suggested that all of the vanadium is located in the blood, whereas the experiments of Kobayashi and of Bertrand localized the majority of the vanadium in other parts of the animal. In *Ascidia mentula* Bertrand found $\frac{2}{3}$ of the vanadium in the tunic, yet Kobayashi was able to establish only traces of vanadium existing in the outer covering of Japanese ascidians.

The dynamics of the concentration of vanadium from sea water by these marine animals has received scant attention. Bertrand describes the situation as a complete mystery and states that the vanadium could come neither from sea water, which is very poor in vanadium, nor from vanadium-impoverished marine muds in the vicinity of the ascidians. He invites research on plankton as a potential source of vanadium. Rankama and Sahama (1950) suggest, however, that the vanadium is collected by ascidians directly from sea water.

Thus, from a biogeochemical standpoint it seemed desirable to investigate the more intimate details of the concentration of vanadium by marine organisms. The

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[2] A comprehensive survey of the biogeochemistry of vanadium has been prepared by D. Bertrand (1950).

present investigation was undertaken in order to attempt to follow the transfer of vanadium from the sea into the various parts of the body of ascidians. A knowledge of the mode of uptake and its histological distribution might shed light upon a possible role played by the vanadium.

To lay the groundwork for these investigations, chemical analyses of dissected portions of some local ascidians were made. This work was intended to establish more definitely the sites of vanadium concentration. A limited number of analyses were made on the total vanadium content of some local species for inclusion in Webb's phylogenetic table.

Cyclotron-produced radioactive vanadium, free from any inert vanadium carrier, was utilized to follow the assimilation of the element. Radioautographs were made to confirm the results of the chemical analyses and also to point out any smaller loci of vanadium concentration not detected by the less sensitive chemical method.

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Chemical Analytical Techniques

Specimens of *Ciona intestinalis* were collected from floats in Mission Bay, San Diego, California. The *Ascidia ceratodes* specimens were obtained from dredgings in Tamales Bay, Marin County, north of San Francisco, California.

Table I

Vanadium content of Ciona intestinalis

Specimen A			Specimen B		
Sample	Dry Wt.	Vanadium	Sample	Dry Wt.	Vanadium
	grams	γ		*grams*	γ
Tunic	0.1629	0.0	Tunic	0.3317	0.0
Mantle	—	—	Mantle	0.0709	0.0
Ovary	0.0330	2.5	Ovary	0.0241	0.0
Heart	0.0084	0.0	Heart	0.0113	0.0
Incurrent Syphon	—	—	Incurrent Syphon	0.0368	4.0
Excurrent Syphon	—	—	Excurrent Syphon	0.0228	0.0
Esophagus	—	—	Esophagus	0.0014	0.0
Branchial Basket	—	—	Branchial Basket	0.0241	0.0
Gut			Gut		
Anterior Half	0.0517	19.7	Anterior Half	0.1116	21.9
Posterior Half	0.1375	8.9	Posterior Half	0.1252	37.8
Stomach	0.0543	18.8	Stomach	0.0586	9.2
Rest of Specimen and Rinse	0.2299	27.0	Rest of Specimen and Rinse	—	—
Rinse		—	Rinse	0.2955	19.6
Total	0.6777	76.9	Total	1.1140	92.5
Date Ciona Obtained	24 July 1950		Date Ciona Obtained	20 April 1950	
Wet Weight	19.0 grams		Wet Weight	19.0 grams	
Length	8.0 cm		Length	6.5 cm	

TABLE II

Total vanadium contents of some ascidians

Ascidian	Parts Vanadium Per Million
Styella montereyensis	0
Styella bonharti	0
Euherdmania claviformis	475
Ciona intestinalis	100

The specimens were cleaned of adhering dirt and sessile organisms by the light application of a tooth brush. After dissection the individual samples were placed in weighed 50 ml. Pyrex centrifuge tubes. The tube and contents were dried overnight at 110° C., cooled in a desiccator, and weighed to determine the "dry weight" of the sample.

The organic matter was removed by a wet digestion as follows: to the sample-containing tube, 3.0 ml. of a solution of one part concentrated sulfuric acid to three parts of 60 per cent perchloric acid were added. The tube was heated gently with a micro-burner until the sample was nearly completely digested. Stronger heat was then applied to facilitate the complete removal of the perchloric acid. The sulfuric acid residue was diluted to 40 ml. with distilled water, and any remaining solids (usually diatom frustules) were removed by centrifugation.

The vanadium in the clear solution was determined by a spectrophotometric analysis of the vanadium 8-hydroxyquinolate in iso-amyl alcohol according to the method of Bach and Trelles (1941). The samples were read at 480 mμ in 50 mm. cells on a Beckman Model DU Spectrophotometer.

The results of two typical analyses of *Ciona intestinalis* are summarized in Table I. The vanadium concentrations for other ascidians collected during the course of these investigations are listed in Table II.

Relatively high concentrations of vanadium occur in the gut. The gut tube was arbitrarily dissected into four serial lengths. It was found that the highest vanadium concentrations per unit weight were associated with that part of the gut nearest the stomach, and with the segment adjoining the anus (Table III). Vanadium was normally detected in the stomach by these chemical analyses.

Vanadium was also found in fecal pellets in concentrations ranging from 0 to 800 parts per million by weight. Fecal pellets discharged by the animal during its relaxation before dissection, or dislodged during the dissection, may account for the high vanadium content of the rinse water as noted in Table I.

TABLE III

Concentration of vanadium in gut sections of Specimen B

Section	Length	Dry Weight	Vanadium
1 Next to Stomach	10 mm.	0.0601 gr.	19.3γ
2 Next segment	10 mm.	0.0515 gr.	2.6γ
3 Next segment	10 mm.	0.0601 gr.	11.8γ
4 Next to Anus	10 mm.	0.0651 gr.	26.0γ

Feeding Experiments Utilizing Radioactive Vanadium

V^{48}, a positron and gamma ray-emitting nuclide with a half-life of sixteen days, was prepared by the deuteron bombardment of a titanium target according to the reactions $Ti^{47}(d,n)V^{48}$ and $Ti^{48}(d,2n)V^{48}$. The bombardments were made at the 60-inch Crocker Cyclotron in Berkeley, California, with 20 Mev deuterons for one hour with a total irradiation of 7 micro-ampere hours. The titanium target was composed of one gram of the C.P. metal powder supported on a copper plate covered with a 0.25 mil tantalum foil.

The sub-micro amounts of V^{48} were extracted from the titanium powder to give a carrier-free saline solution of radioactive vanadate, following the method of Haymond, Maxwell, Garrison and Hamilton (1950). The nuclide V^{48} was identified by its half life and by aluminum absorption curves to establish the 0.7 Mev positron reported by Peacock and Deutsch (1946).

Individual ascidians were then placed in aerated Pyrex containers which held two liters of sea water. These aquaria were kept in a cold room maintained at a temperature of $12° \pm 2°$ C.

The sea water was normally filtered before use. In preliminary experiments it was found that the radioactive vanadium was adsorbed on particulate matter in non-filtered sea water and was thus removed from solution. The water was filtered through either Whatman No. 42 paper or a diatomaceous earth column. The radioactive vanadium was added after filtration.

The activity of the solution was assayed daily by evaporating under an infrared lamp a one-ml. aliquot of the sea water in a steel cup. The activity of the sample was counted under an end-window tube with a window thickness of 1.4 mg./cm^2.

A control aquarium was set up without an ascidian to determine whether there was adsorption of the radioactive vanadium on the walls of the container. No measureable adsorption was found to take place.

The initial counting rate of the radioactive solution was about 250 counts per minute per ml. of solution. The counter geometry was estimated at 5 per cent for 0.7 Mev positrons from measurements with a calibrated U_3O_8 source and aluminum absorption experiments on the active vanadium. Utilizing the radioactive decay equation,

$$dN/dt = -\lambda N \qquad (1)$$

where N is the number of atoms of the radionuclide present, λ is the decay constant equal to 0.693 divided by the half life, and dN/dt is the instantaneous disintegration rate, 10^{11} atoms of active vanadium are found to exist in one liter of solution initially. This may be compared with the upper limit of vanadium in sea water of 10^{16} atoms per liter as determined spectroscopically by D. Bertrand (1942). It should be noted, however, that the experimentally determined impurity of 0.01 per cent of vanadium in the one gram of titanium metal target introduced about 10^{16} atoms of vanadium when it was diluted to 100 liters in the experiments that were undertaken.

Three possibilities existed for the mode of removal of vanadium from sea water by the ascidians. The first type would be an erratic assimilation in which no consistent variation of the remaining vanadium in the solution with time would be found. In the second case, the ascidians might concentrate constant amounts of

vanadium per unit time, whereby a plot of the vanadium remaining in solution against time would give a linear relationship. Another possible mechanism would involve the ascidians concentrating the vanadium in direct proportion to the amount of vanadium present in the sea water environment. In such a case, a graph of

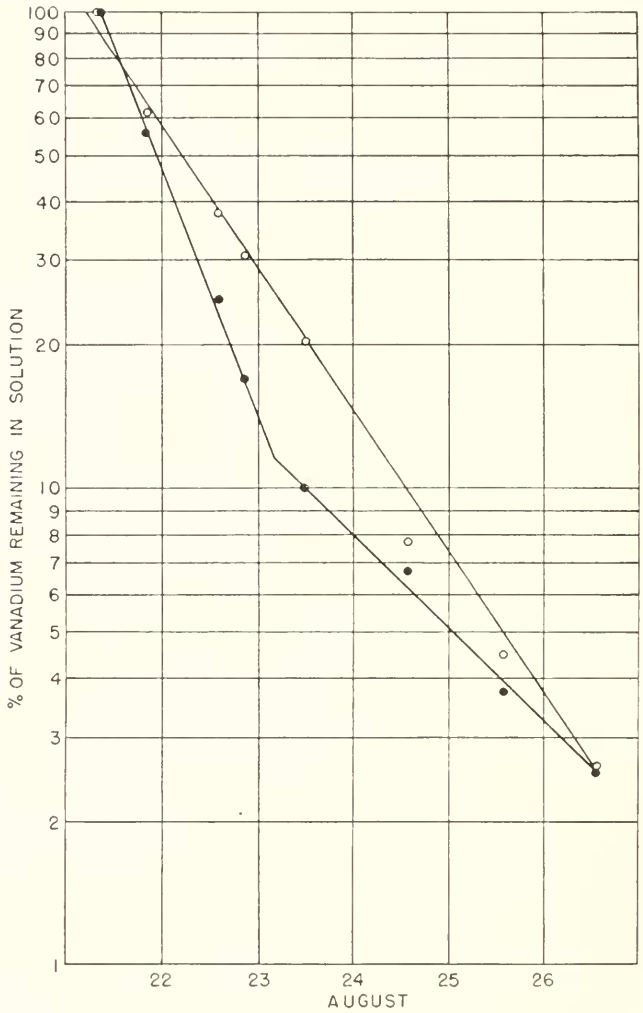

FIGURE 1. Two uptake curves of vanadium by *Ascidia ceratodes*. ○ Experiment 101. ● Experiment 2.

the logarithm of the remaining amount of vanadium in the solution against time should result in a linear relationship. This last mechanism was found to be followed in experiments with *Ascidia ceratodes,* as is illustrated in Figures 1 and 2 where the logarithm of the vanadium remaining in solution (corrected for radioactive decay) is plotted against the time of withdrawal of the aliquot of sea water. In

studies on *Ciona intestinalis* all of the curves obtained could be divided into two straight lines wherein a high initial uptake was followed by an abrupt change to a lower feeding rate. This is illustrated in Figure 2. In 10 experiments on *Ascidia ceratodes,* only two sharp changes were found in each case.

The instantaneous uptake of vanadium by these ascidians may be designated by

$$dN/dt = - VR \qquad (2)$$

where V is the concentration of vanadium at time t in the sea water, N is the total amount of vanadium in the volume A of the sea water, and R is the effective clearance rate of the vanadium from the solution in units of volume per unit time,

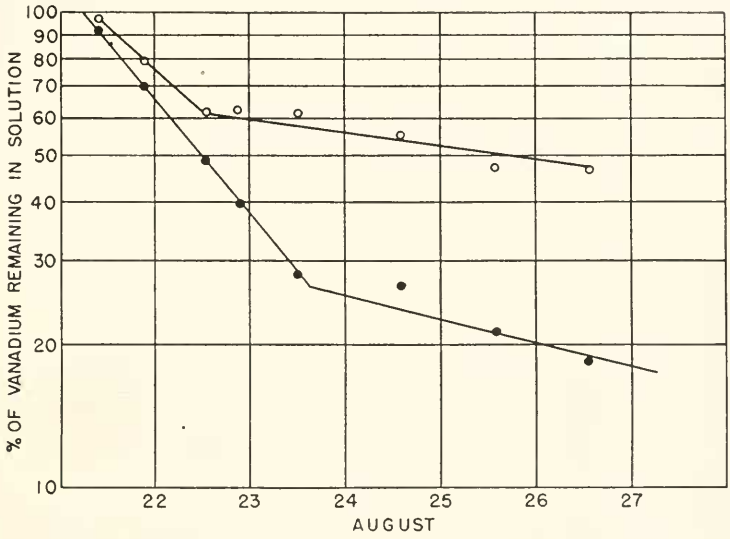

FIGURE 2. Two uptake curves of vanadium by *Ciona intestinalis.* ○ Experiment 7. ● Experiment 5.

assuming a 100 per cent uptake of vanadium. It follows that

$$V = N/A. \qquad (3)$$

Utilizing Equation (3) and integrating Equation (2) from the beginning of the experiment we arrive at

$$V/V_0 = e^{-(R/A)t} \qquad (4)$$

where V_0 represents the initial concentration of vanadium. For the purpose of comparative study we define the "half-time of vanadium in solution" as the time required for a given animal to reduce the vanadium concentration to half of its initial value. The values for the half-time for the 14 experiments conducted are listed in Table IV with the details of each run. Where changes occurred, the initial half-time is given.

The half-time will be a function of the effective adsorbing surface (*i.e.,* the geometry) and the rate at which the water is circulated through the animal. Where

there is a change in the half-time during the course of an experiment, either or both of these parameters may change.

These experiments indicate that the initial transfer of vanadium from the hydrosphere to ascidians occurs by an adsorption process where the adsorbing surface is in an unsaturated state with respect to the adsorption of vanadium at all times, or is maintained in such a state by a constant regeneration of the surface. In either case the number of vanadium ions (or colloidal particles containing vanadium) would determine the number adsorbed at any given time.

To study more closely the dynamics of the vanadium transfer, the sea water in six experiments was enriched in phosphate ion. In the case of *Ascidia ceratodes* a high phosphate level inhibited the uptake of vanadium. This is seen in Experiments 4 and 104 where 50 microgram atoms of phosphate per liter were added

TABLE IV

Half-times of vanadium in solutions containing ascidia

Experiment	Animal	Weight in Grams	Half-time Hours	Filter Treatment	Remarks
1	*A. ceratodes*	20.5 10.0	10	None	Two animals
2	*A. ceratodes*	13.5	14	Whatman 42	
3	*A. ceratodes*	23.0	24	Whatman 42	5μgr. at. $PO_4^{\equiv}$/liter
4	*A. ceratodes*	23.2	65	Whatman 42	50μgr. at. $PO_4^{\equiv}$/liter
101	*A. ceratodes*	29.8	24	None	
102	*A. ceratodes*	38.7	12	Whatman 42	
103	*A. ceratodes*	36.8	6	Whatman 42	5μgr. at. $PO_4^{\equiv}$/liter
104	*A. ceratodes*	30.9	48	Whatman 42	50μgr. at. $PO_4^{\equiv}$/liter
9	*A. ceratodes*	35.0	14	Silica column	
10	*A. ceratodes*	27.1	37	Silica column	
5	*C. intestinalis*	19.6	29	None	
6	*C. intestinalis*	25.4	22	Whatman 42	
7	*C. intestinalis*	25.6	40	Whatman 42	5μgr. at. $PO_4^{\equiv}$/liter
8	*C. intestinalis*	47.6	18	Whatman 42	50μgr. at. $PO_4^{\equiv}$/liter

initially to the sea water. In these two experiments the greatest half-times were observed. Five microgram atoms of phosphate per liter had little effect upon the uptake as is noted in Experiments 3 and 103. Since pentavalent vanadium ion can resemble phosphate ion chemically in solution, these experiments indicate that the phosphate ion competed successfully with the vanadium in the uptake process, and that vanadium in an ionic form rather than a colloidal vanadium is adsorbed. However, the solution chemistry of elements in concentrations in the level of vanadium in the sea and in our experiments has not been developed to the extent to give an unqualified answer.

PUMPING RATE STUDIES ON CIONA INTESTINALIS

To determine the efficiency of transfer of the vanadium from the sea water to the animals, investigations concerning the effective pumping rates of *Ciona intestinalis* were conducted. In each experiment a single animal was placed in a small plastic aquarium two inches deep (Fig. 3). A continuous flow of sea water

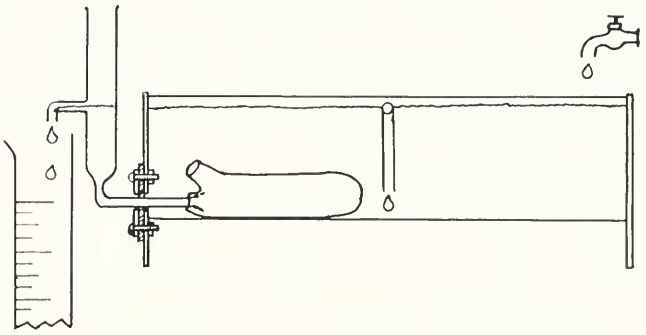

FIGURE 3. Aquarium used for pumping rate studies of ascidians.

was maintained and the water level was kept constant by means of drains placed at the top of the aquarium. A Pyrex glass tube of 4 mm. outer diameter with a 6 mm. outer diameter bulb at one end was inserted in the opened exhale syphon of the ascidian with great care to avoid injury to the animal. The animals soon relaxed with such an arrangement. The ascidian, with inserted tube, was then placed in the aquarium and the tube connected to a second glass tube which entered the aquarium through a rubber gasket and discharged the water outside of the aquarium at the same level as the drains. There was therefore no hydrostatic head to aid

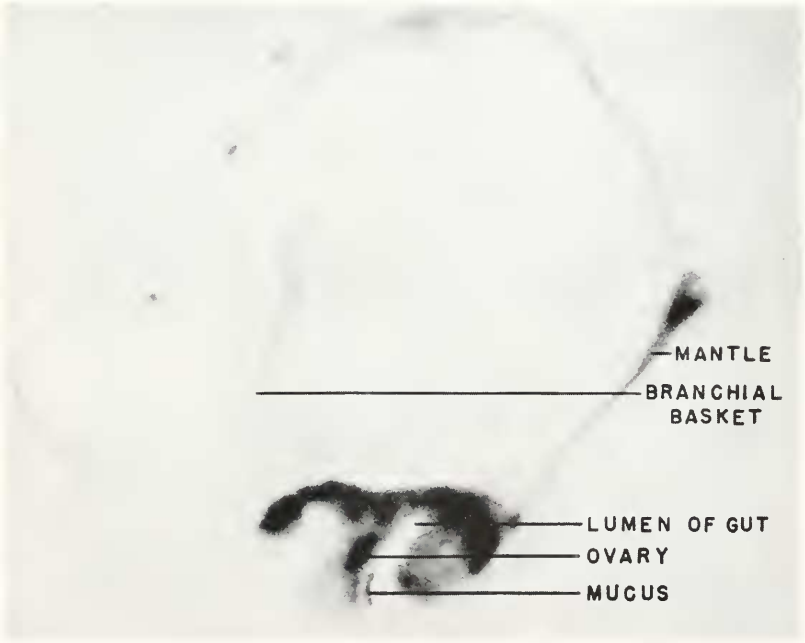

FIGURE 4. Radioautograph of *Ascidia ceratodes* used in Experiment 9. Concentration of vanadium is seen in ovary, gut wall, and in the mucus in the gut. Fifteen day exposure.

or hinder the ascidian in its normal pumping functions. The animals pumped at a constant rate after the tube had been inserted for two hours. The animals seemed to seal their junction to the tube by means of mucus and muscular tonus.

For an ascidian with a wet weight of 25 grams, pumping rates of about two liters per hour were observed. This result compares favorably with the pumping studies of Jørgensen (1949) on *Ciona intestinalis*; using a different technique, he obtained values of the same order of magnitude.

Radioautographic Studies

At the completion of the uptake studies with radio-vanadium the animals were sacrificed for radioautographic analysis. Both *Ascidia ceratodes* and *Ciona intestinalis* were fixed in Bouin's solution, imbedded in paraffin, and sectioned at about 100 micra. Survey autographs were obtained with Eastman No-Screen X-Ray film with exposure times up to 30 days. Figures 4 and 5 show two typical transverse sections.

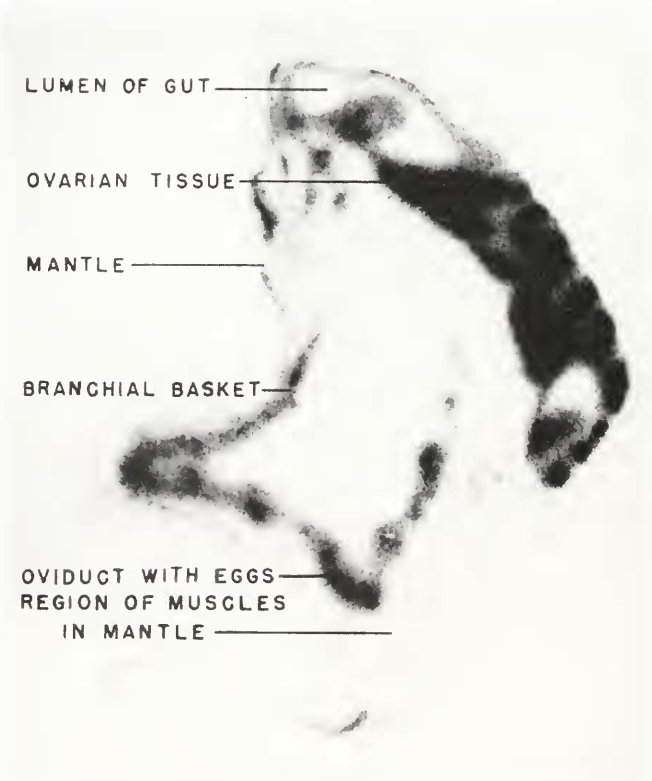

Figure 5. Radioautograph of *Ascidia ceratodes* used in Experiment 102. Concentration of vanadium is seen primarily in ovary, gut wall, oviduct (eggs), and branchial basket, with very small amounts in the mantle. No vanadium concentration was found in the muscles of the mantle. Exposure time of 15 days.

The autographs confirmed the chemical analyses on *Ciona intestinalis,* showing high concentrations in the gut wall and in the ovary, and showed similar local concentrations in *Ascidia ceratodes.* The vanadium concentration in the ovaries of both species is primarily in the follicles. The vanadium was still in the mature eggs when they were extruded from the ovary into the peribranchial cavity.

There is some concentration of the vanadium in the branchial basket of both species, though sometimes not in very great amounts. This enrichment may be due to the blood vessels in this structure, as high concentrations of vanadium in blood vessels were observed. The mantle and test showed very little vanadium and the muscles of the mantle displayed no evidence of this element. Vanadium appeared in the mucus of the branchial basket and in an isolated bit of mucus in the gut as is shown in Figure 4. The absence of high amounts of vanadium in the branchial region may have been due to washing away of the mucus sheet during the fixing procedure or to the constant renewal of fresh mucus by the living animal.

Discussion

The uptake experiments establish that vanadium can be concentrated directly from solution by the two species of ascidians used in this work. To obtain an idea of the efficiency of this adsorption process, we may compare the effective clearance rate of vanadium with the pumping rates for *Ciona intestinalis.* By substitution in Equation (4) of A = 2 liters/hour, $V/V_0 = \frac{1}{2}$, and the half time = 30 hours (the average from Experiments 5, 6, and 7), we arrive at an effective clearance rate of vanadium of 0.05 liters per hour. Compared with the observed pumping rate of 2 liters an hour, the efficiency of uptake of vanadium is of the order of $2\frac{1}{2}$ per cent. This is the value for animals which were unfed, since food in the form of particulate matter could not be introduced without adsorbing vanadium from solution and invalidating the results.

The extraordinary biological specificity of the adsorbing surface of the ascidians for the vanadium in aqueous solution remains an unsolved problem of high biochemical interest. This selectivity is undoubtedly related to the unique ionic structure and properties of vanadium. The mucus sheet fulfills the requirement of a constantly renewed surface. The high vanadium content of the mucus, as shown by the autographs, in the branchial basket and in the gut, points to this sheet as the adsorbing surface. The sharp discontinuities in the uptake curves may be explained by chemical or area changes in the adsorbing surface.

The high concentrations of vanadium in the ovarian follicles, as shown in the autographs, suggest that this element may become part of a metabolic system that is present from the beginning in the developing embryo. Further work utilizing more concentrated radioactive vanadium solutions and thinner autograph specimens should reveal more on the intimate histology of vanadium in these animals.

We may now attempt to account for the high amounts of vanadium found in ascidians. In the case of *Ciona intestinalis* which contains about 100 micrograms of vanadium in a mature 20 gram animal, a minimum of 450 liters of sea water may be effectively cleared of vanadium in a year. This is equivalent to about 45 micrograms of vanadium. Further, we must consider that vanadium and many other elements are ionically or colloidally adsorbed on the particulate matter of the oceans. This phenomenon presented itself in the preliminary feeding experiments.

Thus, a more concentrated source of vanadium is available to these filter feeders. A final potential store of vanadium lies in the plankton of the oceans, a source of ultimate food for these filter feeding animals.

Summary

1. Chemical analyses made on *Ciona intestinalis* indicated the vanadium to be localized in the gut region and the ovaries. These sites of assimilation were confirmed by radioautographs utilizing cyclotron-produced radioactive vanadium.

2. The total vanadium content of four local species of ascidians was determined. The uptake of radioactive vanadium from sea water indicated that *Ciona intestinalis* and *Ascidia ceratodes* are able, by means of an adsorption mechanism, to concentrate vanadium directly from sea water. The efficiency of assimilation was of the order of $2\frac{1}{2}$ per cent for unfed animals.

3. Pumping rate of water by *Ciona intestinalis* was determined by a direct method. It was concluded that sea water and its particulate constituents could furnish the necessary vanadium demanded by *Ciona intestinalis*.

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